Final Report
on Work Done Under Contract Nonr-2437(00) on
ICE FORMATION IN AQUEOUS MEDIA OF BIOLOGICAL INTEREST

B. J. Luyet
American Foundation for Biological Research, Madison, Wis.

This program deals, on the whole, with the changes which take place in aqueous media, mostly solutions, when their temperature is lowered and raised at various rates to various end points. Particular emphasis is placed on the effects of rapid cooling to very low temperatures in conditions under which a stable equilibrium cannot be reached, and on the effects of a relatively slow rewarming during which the equilibrium is reestablished. The information obtained can be classified into three categories: (1) methods, (2) changes observed, (3) special problems; these are presented in three sections in the table of contents below.

The findings were published in 9 papers (some 125 pages) and in 7 abstracts of communications made at meetings of the Society for Cryobiology, the Biophysical Society and the American Physiological Society. The papers (numbered 1 to 9) and the abstracts (numbered A1 to A7) are listed hereafter and the subject-matter treated is indicated in the table of contents by the numbers in the columns on the right side.

Table of Contents:	Papers:	Abstracts:
1. Methods for obtaining rapid cooling and for determining cooling rates; rates actually obtained	2	A2,A3 A4,A6
2. Changes encountered when aqueous solutions are cooled and rewarmed at various rates		
(a) Nucleation		A5
(b) Vitrification and glass transition	6,7,8	
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3. Special problems: state of water in tissues of hibernators and role of magnesium in their blood	4,9	

TITLES OF PAPERS AND ABSTRACTS PUBLISHED
AND BIBLIOGRAPHICAL REFERENCES

(A) Papers

(1) B. Luyet, C. Kroener and G. Rapatz. Detection of Heat of Recrystallization in Glycerol-Water Mixtures. Biodynamica, 8, pp. 73-80, 1958.

(2) B. J. Luyet. A Method for Increasing the Cooling Rate in Refrigeration by Immersion in Liquid Nitrogen or in Other Boiling Baths. Biodynamica, 8, pp. 331-352, 1961.

(3) B. Luyet, J. Tanner and G. Rapatz. X-Ray Diffraction Study of the Structure of Rapidly Frozen Gelatin Solutions. Biodynamica, 9, pp. 21-46, 1962.

(4) B. J. Luyet. On the State of Water in the Tissues of Hibernators. Ann. Acad. Sci. Fennicae, Ser. A, pp. 297-309, 1964.

(5) B. Luyet. The Problem of Structural Instability and Molecular Mobility in Aqueous Solutions "Solidified" at Low Temperatures. Biodynamica, 10, pp. 1-32, 1966.

(6) B. Luyet and C. Kroener. The Temperature of the "Glass Transition" in Aqueous Solutions of Glycerol and Ethylene Clycol. Biodynamica, 10, pp. 33-40, 1966.

(7) C. Kroener and B. Luyet. Discontinuous Change in Expansion Coefficient at the Glass Transition Temperature in Aqueous Solutions of Glycerol. Biodynamica, 10, pp. 41-45, 1966.

(8) C. Kroener and B. Luyet. Formation of Cracks During the Vitrification of Glycerol Solutions and Disappearance of the Cracks During Rewarming. Biodynamica, 10, pp. 47-52, 1966.

(9) R. Masat and B. J. Luyet. The Effect of Magnesium Ion on Blood-Clotting Times in an Active-Phase Hibernator and a Non-Hibernator. Comp. Biochem. Physiol., 21, 1967.

(B) Abstracts

(A1) B. Luyet and G. Rapatz. On Eutectic Freezing in Biological Fluids. Abstract No. 377, Eighty-Second Meeting of the Amer. Physiol. Soc., Atlantic City, N. J., April 13-14, 1959. Feder. Proc., Vol. 18, p. 96.

(A2) B. Luyet and C. Kroener. The Highest Obtainable Cooling Velocities. Abstract L7, Fourth Annual Meeting of the Biophysical Society, February 24-26, 1960, Philadelphia, Pennsylvania.

(A3) B. Luyet. Toward Higher Freezing Rates. Abstract SE6, Fifth Annual Meeting of the Biophysical Society, February 16-18, 1961, St. Louis, Missouri.

(A4) A. P. MacKenzie, L. W. Evers and B. J. Luyet. Effects of the Thickness of an Insulating Coat on the Cooling Velocity of Heat-Conductive Objects Immersed in Liquid Nitrogen. Abstract TC8, Sixth Annual Meeting of the Biophysical Society, February 14-16, 1962, Washington, D. C.

(A5) B. J. Luyet and C. Kroener. Grain Size in Blood Plasma Frozen under Various Conditions. Abstract No. 6, Second Annual Meeting of Soc. for Cryobiol., Madison, Wisconsin, Aug. 2-4, 1965. Cryobiology, 2, p. 8.

(A6) A. P. MacKenzie, C. Kroener and B. J. Luyet. Rapid Cooling by Means of Pressurized Liquid Nitrogen. Abstract No. 26, Second Annual Meeting of Soc. for Cryobiol., Madison, Wisconsin, Aug. 2-4, 1965. Cryobiology, 2, p. 17.

(A7) C. Kroener and B. J. Luyet. Determination of the Heat Released in the Rewarming of Rapidly Cooled Solutions of Polyvinylpyrrolidone. Abstract No. 40, Second Annual Meeting of the Soc. for Cryobiol., Madison, Wisconsin, Aug. 2-4, 1965. Cryobiology, 2, p. 22.

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Basile J. Luyet, Calvin Kroener, Gabriel L. Rapatz, Alan P. MacKenzie, J. Tanner, R. Masat, L. W. Evers

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13. ABSTRACT

~~The program of the~~ research reported here emphasized the importance of the cooling rates in the preservation of biological material in the frozen state (such as would be the case, for example, in the preservation of blood).—The cooling rates obtained by immersion of specimens in various refrigerants, including liquid nitrogen under pressure or at its freezing point, and liquid helium II, were measured. The method of increasing the rate of cooling of an object immersed in a boiling refrigerant by coating the object with a vapor-nucleating substance was investigated; in some cases the rate could be increased 23 times.—The study of the structural instability and molecular mobility in rapidly cooled aqueous solutions which are partly crystalline and partly amorphous furnished information on changes occurring at the temperatures of (a) the "glass transition", (b) devitrification, (c) recrystallization (of which 4 kinds were distinguished), and (d) eutectic melting. These changes were investigated by various methods, in particular, dilatometry, calorimetry and X-ray diffraction.—Tentative applications of our basic studies to biological materials included determinations of the water-binding capacity of the tissues of hibernating animals.

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